Effect of Carnosine on Growth Performance, Carcass Characteristics, Meat Quality and Oxidative Stability in Broiler Chickens

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The present study was conducted to evaluate the effect of dietary carnosine on growth performance, carcass characteristics, meat quality, and oxidative stability of muscle and blood in Arbor Acres broilers. A total of 180 1-d-old male broiler chicks were randomly assigned to groups: group A, control feed; group B, 0.30% carnosine feed during d to d 21; group C, 0.50% carnosine feed during d 22 to d 42. Birds were sacrificed at d 42. Live body weight, muscle color (L*, a*, b*), pH values at 0, 2, and 24h postmortem, meat shear force value (SFV), water loss rate, the amount of thiobarbituric acid reactive substances (TBARS), and total antioxidant capability (TAOC) in the blood and the muscle tissue were measured. Results showed that the growth performance of the chickens was not affected by dietary carnosine. The breast muscle weight and thigh muscle weight of group B and the thigh muscle weight of group C was significantly higher than that of group A (P < 0.05). The SFV of the breast and the thigh muscle of group C and the SFV of the breast muscle of group B was significantly lower than that of group A (P < 0.05). Neither the muscle color nor the pH values at 0, 2, and 24 h postmortem were affected. The dietary carnosine decreased the TBARS values and increased the TAOC both in the blood and muscle significantly (P < 0.01). These results suggest that dietary carnosine improves chicken meat quantity and quality.

Key words: broiler, carnosine, meat quality, oxidative stability

Introduction

Lipid oxidation is one of the main factors that cause quality deterioration in meat and meat products. Lipid oxidation leads to meat spoilage and has been reported to cause adverse changes in poultry meat quality (Kennedy et al., 2005). For example, the color and flavor of meat are both dependent on oxidative mechanisms (Ouali et al., 2006).

The quality of chicken meat can be improved by antioxidants in feed because most antioxidants are incorporated within cell membranes and protect tissues against oxidation from reactive oxygen species. However, pressure to reduce artificial additive use in foods has led to attempts to increase meat stability by dietary strategies in recent years (Morrissey et al., 1998). Thus, dietary nutrients which have strong anti-oxidative capacity and minimal or no negative effects on meat quality received much more attention from scientific researchers. Consequently, it has been found that many feed additives, such as α-tocopherol, β-carotene, ascorbic acid, oregano, and grape pomace, are effective in reducing lipid oxidation in meat and meat products to extend the shelf life (Young et al., 2003; Guo et al., 2006; Brenes et al., 2008).

The natural dipeptide L-carnosine (β-Ala-His) exhibits antioxidant activity and can be used as an antioxidant in food products. Inhibition of oxygen consumption, conjugated dienes, TBARS, and total volatile compound formation by carnosine demonstrates that it actually inhibits the iron-induced peroxidation of muscle phospholipids (Kansci et al., 1997). Morrissey et al. (1998) reported that carnosine in combination with dietary vitamin E supplementation improves lipid stability in processed meats. In addition, carnosine has the potential to improve meat quality and the multiplicity of other biologic activities, especially for their natural and safety with minimal toxicity and side effects (Guiotto et al., 2005; Tomonaga et al., 2005). However, there is very limited information regarding the effect of dietary carnosine on the oxidative stability and meat quality in chicken.

The objectives of this experiment were to investigate the effect of dietary carnosine on growth performance, meat quality and oxidative stability in broiler chickens. Lu et al. (2006) found that muscle fiber diameter of broilers administered carnosine via drinking water was higher than
that of the control group during weeks 1 to 3 but lower than that of the control group during weeks 4 to 6. We followed this study and designed two supplementation periods: 1–21 d and 22–42 d.

**Materials and Methods**

**Birds and Housing**
A total of 180 1-d-old male Arbor Acres broilers were selected to investigate the effect of dietary carnosine on growth performance, meat quality and oxidative stability. The birds were randomly assigned to 3 treatments each with 6 pens of 10 birds. Broilers were vaccinated for Newcastle disease and infectious bronchitis disease, on d 7 and d 21, respectively. A 24h lighting schedule was carried out during the first 3 days, and 23h lighting with 1h darkness was used from d 4. Mean air temperature of the animal chamber was about 35°C during the first week, and then decreased gradually to a constant temperature 25°C. Feed and water were freely available to all birds. The entire experiment lasted 42 days.

**Experimental Design and Treatments**
The experiment had a completely randomized block design. Three dietary treatments were considered: group A (control group), group B (0.5% carnosine from d 1 to d 21) and group C (0.50% carnosine from d 21 to d 42). Compositions and nutrient levels of the 3 treatment diets for starters (1–21 d) and growers (22–42 d) are shown in Table 1. All nutrient contents met or exceeded the NRC recommendations (NRC, 1994). In our preliminary experiment, 0.5% carnosine-containing feed improved the tenderness of the breast muscle in broiler chickens. Thus, this dose (0.5%) was used in the present study.

**Sample Collection**
The body weight and feed intake of each pen was measured at d 22 and d 42. The calculated average values of the body weight gain, feed intake and the ratio of feed to gain of each bird per day were used for data analysis. One bird from each pen with a body weight close to the average of the pen was anesthetized by intraperitoneal injection of sodium pentobarbitone and sacrificed by exsanguination 12 h after feed and water deprivation. Blood was collected from the wing vein, and the serum was prepared and stored at −30°C until assay. After measuring the carcass weight, breast and thigh muscle from both sides of the carcass were skinned and deboned for the measurements of carcass traits, muscle color (L*, a*, b*), pH value, tenderness, water loss rate and the oxidative stability values.

**Table 1. Composition and nutrient content of experimental diets in different growing phase**

<table>
<thead>
<tr>
<th>Age Group</th>
<th>1–21 d</th>
<th>22–42 d</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
<td>B</td>
</tr>
<tr>
<td>Ingredients (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Corn</td>
<td>46.90</td>
<td>46.90</td>
</tr>
<tr>
<td>Corn gluten meal</td>
<td>5.70</td>
<td>5.70</td>
</tr>
<tr>
<td>Extruded soybean</td>
<td>20.00</td>
<td>20.00</td>
</tr>
<tr>
<td>Soybean meal</td>
<td>20.00</td>
<td>20.00</td>
</tr>
<tr>
<td>Limestone</td>
<td>1.70</td>
<td>1.70</td>
</tr>
<tr>
<td>Dicalcium phosphate</td>
<td>1.20</td>
<td>1.20</td>
</tr>
<tr>
<td>Salt</td>
<td>0.30</td>
<td>0.30</td>
</tr>
<tr>
<td>Corn oil</td>
<td>2.70</td>
<td>2.70</td>
</tr>
<tr>
<td>Premix2</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>Bentonite</td>
<td>0.50</td>
<td>0.50</td>
</tr>
<tr>
<td>Carnosine</td>
<td>0.50</td>
<td>0.50</td>
</tr>
<tr>
<td>Total</td>
<td>100.00</td>
<td>100.00</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Nutrient composition</th>
<th>1–21 d</th>
<th>22–42 d</th>
</tr>
</thead>
<tbody>
<tr>
<td>ME (MJ/kg)</td>
<td>13.33</td>
<td>13.33</td>
</tr>
<tr>
<td>CP (%)</td>
<td>22.60</td>
<td>22.60</td>
</tr>
<tr>
<td>Ca (%)</td>
<td>0.99</td>
<td>0.99</td>
</tr>
<tr>
<td>Total phosphorus (%)</td>
<td>0.64</td>
<td>0.64</td>
</tr>
<tr>
<td>Available phosphorus (%)</td>
<td>0.44</td>
<td>0.44</td>
</tr>
<tr>
<td>Methionine (%)</td>
<td>0.50</td>
<td>0.50</td>
</tr>
<tr>
<td>Methionine + cystine (%)</td>
<td>0.90</td>
<td>0.90</td>
</tr>
<tr>
<td>Lysine (%)</td>
<td>1.12</td>
<td>1.12</td>
</tr>
</tbody>
</table>

1 Group A (control), group B (0.5% carnosine added on d 1 to d 21) and group C (0.5% carnosine added on d 22 to d 42).
2 The vitamin and mineral premix supplied the following per kilogram of diet: vitamin A, 15,000 IU; cholecalciferol, 3,000 IU; vitamin E (DL-alpha-tocopheryl acetate), 20 IU; vitamin K, 2.16 mg; thiamine, 2.16 mg; riboflavin, 8.00 mg; pyridoxine, 4.41 mg; vitamin B12, 0.02 mg; calcium pantothenate, 25.58 mg; nicotinic acid, 65.95 mg; folic acid, 0.98 mg; biotin, 0.20 mg; Fe, 109.58 mg; Cu, 8.14 mg; Zn, 78.04 mg; Mn, 105.00 mg; I, 0.34 mg; Se, 0.14 mg; choline chloride, 1,500 mg.
Muscle homogenate preparation

At 24 h after sacrifice, about 1.0 g ground breast and ground thigh muscles were homogenized in 10 ml of cold 0.9% NaCl at 8,000 rpm for 1 min using Ultra Turrax homogenizer (Model T25, Jane and Kunkel IKA-Labortechnick, Staufen, Germany). After the homogenates were centrifuged at 3,000 x g for 10 min at 10°C, the supernatant was stored at −20°C for the further measurement (Lesiów and Xiong, 2003).

The measurement of protein content in muscle homogenate

Protein content of the muscle homogenates was measured by the biuret method using a commercial kit (Jiancheng Bioengineering Institute).

Total antioxidant capability (TAOC) assay

The antioxidant defense system consists of enzymatic and non-enzymatic antioxidants, which are able to reduce Fe^{2+} to Fe^{3+}. Thus, TAOC can be measured by the reaction of phenanthroline and Fe^{3+} using a commercial kit (Nanjing Jiancheng Bioengineering Institute, Nanjing, China) according to the manufacturer’s instructions. 0.1 ml of the sample (serum or 10% muscle tissue homogenate) was mixed with 3.6 ml of compound reagent in the kit, then heated up to 37°C and reacted for 30 min. Subsequently, the tubes were cooled to room temperature for 10 min, and the absorption value was determined at 520 nm using a UV/Vis Spectrophotometer (Model UV-1100, Shanghai MAPADA Instruments Co., Ltd. Shanghai, China). One TAOC unit (U) is defined as the amount of antioxidants required to increase the absorbance (OD) value of the reaction system by 0.01 with 1 ml sample liquid in 1 min at 37°C. The value of TAOC in the muscle homogenate and blood serum was expressed as U per milligram protein and U per milliliter, respectively.

Thiobarbituric acid-reactive substances (TBARS) measurement

The TBARS value was measured with the modified procedure described previously (Ahn et al., 1998; O’Keefe and Wang, 2006). This is an extraction procedure in which homogenized muscle is allowed to react to the thiobarbituric acid reagent. After the sample (200 μl) was mixed with butylated hydroxyanisole (50 μl, 7.2%) and thiobarbituric acid/trichloroacetic acid (TBA/TCA; 2 ml), the mixture was vortexed and then incubated at 95°C water bath for 40 min. Then the sample was cooled in cold water for 10 min and centrifuged for 10 min at 3,000 g. The absorbance of the supernatant was determined at 532 nm using a UV/VIS spectrophotometer (Model UV-1100, Shanghai MAPADA Instruments Co., Ltd. Shanghai, China). The TBARS values were expressed as micromoles of malondialdehyde (MDA) per microgram of muscle protein and micromoles of MDA per milliliter of serum.

Statistical Analysis

The statistical analyses were performed using the general linear model analysis of variance procedure, correlation analysis procedure of Statistical Analysis System (SAS 8.2). Significant effects were further explored using ANOVA to ascertain differences among means. Means were compared using Duncan’s multiple range test method. The significance level was designed as 0.05. The correlative relationships between oxidative stability (TAOC and TBARS) and meat quality (pH, SFV, color and WLR) were performed with the correlation analysis procedure.
Results

Growth Performance
As shown in Table 2, final body weight and body weight gain were not affected by dietary carnosine. Feed intake and feed conversion ratio were also not affected.

Carcass Characteristics
The effects of dietary carnosine on carcass traits are presented in Table 3. Live body weight, carcass weight, eviscerated yield, dressing percentage, and percentage of eviscerated yield were not affected by dietary carnosine. The breast muscle weight, thigh muscle weight, breast muscle percentage, and thigh muscle percentage of group B was significantly higher than that of group A \((P<0.05)\). The thigh muscle weight and the thigh muscle percentage of group C were significantly higher than that of group A \((P<0.05)\).

Meat Quality
As shown in Table 4, there were no significant differences in the pH0, pH2 and pH24 of the breast and thigh muscle among the groups. The SFV of the breast muscle of group B and C was significantly lower than that of group A \((P<0.01)\). The SFV of the thigh muscle of group C was significantly lower than that of group A \((P<0.05)\). The values of hunter \(L^*\) and \(b^*\) in breast muscles and the values of hunter \(a^*\) and \(b^*\) in thigh muscles were not significantly different among groups but the hunter \(a^*\) value of the breast muscle in group B was lower than that of group A. The WLR of breast muscle and thigh muscle was not affected by dietary carnosine.

Oxidative Stability
As shown in Table 5, all TAOC and MDA values in group C were significantly improved in the serum, breast and thigh muscles compared to group A \((P<0.05)\). In group B, TAOC value in the serum and breast muscle and MDA value in the serum and thigh muscle were significantly improved when compared to those of group A \((P<0.05)\).

Correlation Coefficients Between Meat Quality and Oxidative Stability
Pearson correlation coefficients between meat quality and oxidative stability were shown in Table 6. The TAOC of the serum was significantly negatively correlated with the SFV of the breast muscle \((r=-0.511)\). The TAOC of the breast muscle was significantly negatively correlated with the SFV of the breast muscle \((r=-0.611)\). The TAOC of the thigh muscle was significantly positively correlated with pH24 of the thigh muscle \((r=0.551)\), and negatively correlated with the SFV and the hunter \(L^*\) value of the thigh muscle \((r=-0.778, r=-0.507,\) respectively). In contrast, the MDA of the serum was found to be significantly positively correlated with the SFV of the breast and thigh muscle and the hunter \(b^*\) value of the thigh muscle \((r=0.545, r=0.671)\, and \(r=\)

### Table 2. Effect of dietary carnosine on the growth performance of broilers (1d-42 d)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
</tr>
<tr>
<td>Start BW (g)</td>
<td>41.0 ± 1.9</td>
</tr>
<tr>
<td>Final BW (g)</td>
<td>1969.3 ± 64.2</td>
</tr>
<tr>
<td>Weight gain (g/bird per d)</td>
<td>45.9 ± 1.5</td>
</tr>
<tr>
<td>Feed intake (g/bird per d)</td>
<td>92.4 ± 3.3</td>
</tr>
<tr>
<td>FCR</td>
<td>2.01 ± 0.09</td>
</tr>
</tbody>
</table>

Values are mean ± S.D. \((n=6)\) each.

BW, body weight; FCR, feed conversion ratio.

### Table 3. Effect of dietary carnosine on carcass characteristics of broilers at 42 d

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
</tr>
<tr>
<td>Live body weight (g)</td>
<td>2112.2 ± 201.7</td>
</tr>
<tr>
<td>Carcass weight (g)</td>
<td>1922.2 ± 188.7</td>
</tr>
<tr>
<td>Eviscerated yield (g)</td>
<td>1526.0 ± 149.9</td>
</tr>
<tr>
<td>Breast muscle weight (g)</td>
<td>277.33 ± 30.72</td>
</tr>
<tr>
<td>Thigh muscle weight (g)</td>
<td>275.67 ± 31.35</td>
</tr>
<tr>
<td>Dressing percentage (%)</td>
<td>90.97 ± 0.32</td>
</tr>
<tr>
<td>Percentage of eviscerated yield (%)</td>
<td>72.22 ± 0.38</td>
</tr>
<tr>
<td>Breast muscle percentage (%)</td>
<td>18.16 ± 0.67</td>
</tr>
<tr>
<td>Thigh muscle percentage (%)</td>
<td>18.04 ± 0.48</td>
</tr>
</tbody>
</table>

Values are mean ± S.D. \((n=6)\) each.

\(^a,b\)\(^*\) Means within the same row with different superscripts differ significantly \((P<0.05)\).
Table 4. Effect of dietary carnosine on the meat quality of broilers

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
</tr>
<tr>
<td>Breast pH 0</td>
<td>6.09±0.21</td>
</tr>
<tr>
<td>Breast pH 2</td>
<td>5.75±0.18</td>
</tr>
<tr>
<td>Breast pH 24</td>
<td>5.58±0.12</td>
</tr>
<tr>
<td>Thigh pH 0</td>
<td>6.40±0.11</td>
</tr>
<tr>
<td>Thigh pH 2</td>
<td>6.26±0.07</td>
</tr>
<tr>
<td>Thigh pH 24</td>
<td>5.80±0.05</td>
</tr>
<tr>
<td>Breast pH 0–2</td>
<td>0.34±0.09&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Breast pH 0–24</td>
<td>0.51±0.12</td>
</tr>
<tr>
<td>Thigh pH 0–2</td>
<td>0.15±0.08</td>
</tr>
<tr>
<td>Thigh pH 0–24</td>
<td>0.59±0.10&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Breast shear force (N)</td>
<td>28.33±2.65&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Thigh shear force (N)</td>
<td>19.61±1.62&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Breast L*</td>
<td>45.50±1.95</td>
</tr>
<tr>
<td>Breast a*</td>
<td>3.60±0.55&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Breast b*</td>
<td>11.59±1.89</td>
</tr>
<tr>
<td>Thigh L*</td>
<td>50.40±1.61&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>Thigh a*</td>
<td>3.65±0.62</td>
</tr>
<tr>
<td>Thigh b*</td>
<td>14.46±1.43</td>
</tr>
<tr>
<td>Breast water loss rate (%)</td>
<td>26.25±5.26</td>
</tr>
<tr>
<td>Thigh water loss rate (%)</td>
<td>20.82±1.43</td>
</tr>
</tbody>
</table>

Values are mean±S.D. (n = 6 each).<sup>a,b</sup> Means within the same row with different superscripts differ significantly (P<0.05).

Table 5. Effect of dietary carnosine on the oxidative stability of broilers

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
</tr>
<tr>
<td>TAOC serum (U/ml)</td>
<td>12.58±1.72&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>TAOC breast (U/mg protein)</td>
<td>0.09±0.01&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>TAOC thigh (U/mg protein)</td>
<td>0.16±0.03&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>MDA serum (nmol/ml)</td>
<td>6.71±0.91&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>MDA breast (nmol/mg protein)</td>
<td>0.50±0.06&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>MDA thigh (nmol/mg protein)</td>
<td>0.76±0.14&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values are mean±S.D. (n = 6 each).<sup>a,b</sup> Means within the same row with different superscripts differ significantly (P<0.05).

TAOC, total antioxidant capability; MDA, malondialdehyde.

0.463, respectively). The MDA of the breast muscle was found to be significantly positively correlated with the SFV of the breast muscle (r = 0.602), and negatively correlated with pH24 of the breast muscle (r = −0.489).

Discussion

The weight of breast and thigh muscles of group B was significantly higher than that of group A. Furthermore, cumulative feed intake during d 22 to d 42 in group C was 2.87-fold higher than that of d 1 to d 22 in group B, indicating that a larger amount of carnosine was needed in group C. Thus, in terms of cost and the effect of carnosine on quantity of chicken meat, the application of carnosine during d 1 to d 21 should be more practical and economical. Table 3 shows that dietary carnosine significantly increased the percentages of breast and thigh muscle, but the underlying mechanism is not clear. One possible speculation is that insulin has a role in the increase of muscle weight. LeBlanc and Soucy (1994) found that the ingestion of carnosine increased the secretion of insulin in dogs. Insulin enhances the synthesis of protein in chickens (Tesseraud et al., Duchêne et al., 2008). This needs to be researched further.

Generally, muscle pH is associated with meat color. For example, the ultimate pH is known to influence the structure of myofibrils and consequently meat color. However, there was no significant difference in pH values between the groups, although significant differences were found in breast a* value and thigh L* values (Table 4). Therefore, the alterations in meat color by carnosine are
not associated with muscle pH.

Tenderness is considered one of the most important index to the overall meat quality of chicken by consumers (Fletcher, 2002). The three primary sources of variation in tenderness are sarcomere length, connective tissue content, and protein proteolysis (Koohmaraie et al., 2002). The activity of calpastatin and calpains both have the antagonistic and bidirectional regulation function on the tenderness of meat. Ouali (1990) stated that the fragilization of myofibrils can lead to a total transversal disruption of the ultrastructure, which may be due to the synergistic action of lysosomal enzymes (especially cathepsin B and L) and Ca-dependent proteinases, and may also be mediated by the high ionic strength in postmortem muscle. Carnosine can be an endogenous regulator of the sarcoplasmic reticulum Ca-channel activity (Zaloga et al., 1997). Johnson and Hammer (1989) found that an increase of carnosine cause a decrease the inhibitory effect of calpastatin on calpain II. It is therefore possible that dietary carnosine decreases SFV of chicken meat due to the upregulation of calpain II activity.

**TBARS** is a good predictor of meat rancidity and oxidative stability. There is evidence that the antioxidative effect of carnosine is based on free radical scavenging and divalent metal ion chelation (Babizhayev et al., 1994; Lee et al., 1999). In addition, the dietary carnosine increases the glutathione peroxidase (GSHPx) activity in the liver and the kidney in diabetic mice (Lee et al., 2005). Hoac et al. (2006) stated that there was a reciprocal relationship between TBARS formation and GSHPx activity. The variation of GSHPx activity in muscle may be important to the oxidative stability of different meat products (Daun and Åkesson, 2004). In this research, we found that dietary carnosine decreases the formation of TBARS in chicken meat. Further study is needed to clarify the underlying mechanism of the antioxidative effect of dietary carnosine on chicken meat.

In conclusion, dietary carnosine increases breast and thigh muscle weights and improves their tenderness by reducing the SFV. Dietary carnosine also reduced TBARS and increased TAOC in the blood and muscle tissue. These results indicate that dietary carnosine increases meat yield and improves meat quality in broilers. Thus, the natural dipeptide L-carnosine could be a promising candidate for feed additive as a growth promoter and an anti-oxidant.

**Acknowledgments**

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